

Appl. No. 09/402,488

Amdt. Dated October 20, 2004

Reply to Office action of April 29, 2004

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (Previously amended): A method for the preparation of a recombinant polypeptide comprising

a) transforming a host cell with an expression vector comprising:

(1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to

(2) a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a nucleic acid sequence encoding a chymosin pro-peptide, linked in reading frame to (b) a nucleic acid sequence heterologous to the pro-peptide and encoding the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide; operatively linked to

(3) a nucleic acid sequence encoding a termination region functional in said host cell,

b) growing the host cell to produce said fusion protein; and

c) adding a mature form of an autocatalytically maturing aspartic protease, that is capable of cleaving the chymosin pro-peptide, to the fusion protein so that the chymosin pro-peptide is cleaved from the fusion protein to release the recombinant polypeptide.

Claims 2-3 (Canceled).

Claim 4 (Currently amended): The method according to claim 1 wherein said aspartic protease added in step (c) is selected from the group consisting of chymosin, pepsin, HIV-1 protease, pepsinogen, cathepsin and yeast proteinase A.

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Claim 5 (Previously amended): The method according to claim 1 wherein the recombinant polypeptide is hirudin or carp growth hormone.

Claim 6 (Previously amended): The method according to claim 1 wherein the chimeric nucleic acid sequence does not include a sequence encoding a mature form of chymosin.

Claim 7 (Currently amended): The method according to claim 1 wherein the pH is from about 2 to about 7 in step (c).

Claim 8 (Previously amended): The method according to claim 7 wherein the pH is from about 2 to about 4.5.

Claim 9 (Currently amended): The method according to claim 1 wherein step (c) takes place under in vitro conditions.

Claim 10 (Currently amended): The method according to claim 1 wherein step (c) takes place under in vivo conditions.

Claim 11 (Canceled).

Claim 12 (Currently amended): The method according to claim 10 wherein the in vivo conditions are those prevalent in a tissue or bodily fluid of an animal and wherein the tissue or bodily fluid comprises the milk, the stomach, or the gut of said animal.

Claim 13 (Currently amended): The method according to claim 1 wherein the mature form of the aspartic protease added in step (c) is chymosin.

Claim 14 (Currently amended): The method according to claim 1 wherein the aspartic protease added in step (c) is heterologous to the chymosin pro-peptide.

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Claim 15 (Previously amended): The method according to claim 13 wherein the chymosin is added under in vitro conditions.

Claim 16 (Previously amended): The method according to claim 13 wherein the chymosin is added under in vivo conditions.

Claim 17 (Canceled).

Claim 18 (Previously amended): The method according to claim 16 wherein said in vivo conditions take place in a tissue or bodily fluid of an animal and wherein the tissue or bodily fluid is a stomach, gut, or milk of said animal.

Claim 19 (Previously amended): The method according to claim 1 wherein said nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

Claim 20 (Previously amended): A chimeric nucleic acid sequence encoding a fusion protein comprising (a) a nucleic acid sequence encoding a full length chymosin pro-peptide and (b) a nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide.

Claims 21-23 (Canceled).

Claim 24 (Previously amended): The chimeric nucleic acid sequence according to claim 20 wherein the polypeptide is hirudin or carp growth hormone.

Claim 25 (Previously amended): The chimeric nucleic acid sequence according to claim 20 which does not include a sequence encoding a mature form of chymosin.

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Claim 26 (Previously amended): The chimeric nucleic acid sequence according to claim 20 wherein said nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

Claim 27 (Previously amended): The chimeric nucleic acid sequence according to claim 26 wherein the chimeric sequence is as shown in SEQ ID NO:1 or SEQ ID NO:3.

Claim 28 (Previously amended): An expression vector comprising the chimeric nucleic acid sequence according to claim 20 and a regulatory sequence suitable for expression in a host cell.

Claim 29 (Original): A transformed host cell containing an expression vector according to claim 28.

Claim 30 (Original): A transformed host cell containing an expression vector according to claim 28 wherein the host cell is a bacterial cell, a fungal cell, a plant cell or an animal cell.

Claims 31-40 (Canceled).

Claim 41 (Previously amended): A composition comprising a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a first nucleic acid sequence encoding a full length chymosin pro-peptide and (b) a second nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide.

Claim 42 (Canceled).

Claim 43 (Previously amended): The composition according to claim 41 wherein the nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

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Claim 44 (Previously amended): The composition according to claim 41 wherein said chimeric nucleic acid sequence does not include a sequence encoding a mature form of chymosin.

Claim 45-47 (Canceled).